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Abstract

Cardiovascular diseases are a major problem nowadays and their incidence is only increasing. Fruits and vegetables have long been known to have antioxidant properties, but recently chocolate has been the focus of much research as cocoa appears to have similar qualities. *Theobroma cacao*, the plant that cocoa is derived from has been found to be a rich source of flavanols, which may protect against cardiovascular diseases. A literature review was carried out and the main ways in which chocolate may contribute to cardiovascular health are presented; these are the antioxidant, anti-inflammatory, and anti-platelet effects. The lipid profile may also be affected but is still a current topic of debate. The modes of action of the flavanols are summarised, but they are still hypotheses and the exact mechanisms are still unknown. Nevetheless the vast amount of epidemiological evidence suggests a definite link between cocoa from chocolate and cardiovascular health. As chocolate is a popular product sold worldwide, greater research into this area will definitely be beneficial.

Contents

1. Introduction

Chocolate is a well-known, popular food all around the world, and is unique in that it is solid at room temperature yet easily melts in the mouth. Although it is usually sweet and quite calorific, recent studies have shown epidemiological links between the flavanols in chocolate and cardiovascular health. Flavanols are also found in fruit, vegetables, tea and red wine, but cocoa contains much more per weight (Lee *et al* 2003). The exact mechanism of the flavanols and metabolism of chocolate is still unknown, but we know that flavanols have antioxidant, anti-inflammatory and anti-platelet effects which assist in healthy endothelial function, and subsequently cardiovascular health. The aim of this paper is to present the current hypotheses relating to the mechanisms of the flavanols in chocolate, as well as any other benefits that it may have.

2. Method

As research into the link between chocolate and cardiovascular health is a very current, effort was put into using literature that was published as recently as possible. The majority are peer-reviewed journal articles. Articles published in the last 10 years were focused on, but sometimes the author referred to works created more than 10 years ago, and those were still used unless newer articles showing different results were found. Government websites were used for information regarding specific regulations on chocolate, and the World Health Organisation (WHO) website was used for official statistics relating to cardiovascular diseases.

3. Brief background of chocolate

3.1 History

Chocolate is a popular food that is available all over the world now, but has its roots in Central and South America. It comes from the plant *Theobroma cacao* native to the Americas. The original method of

consumption of the cocoa bean however, was very different from what we have today. The Aztecs and Mayans consumed it as a bitter drink with the addition of spices, and it was considered a drink of the Gods (Engler & Engler 2006). The Spanish brought cocoa to Europe, but it did not become a mainstream item until the Industrial Revolution in the late 18th Century, when the development of mass production techniques lowered the cost of production. The first solid chocolate was produced in England in 1847, and slowly gained popularity until its sales overtook that of drinking chocolate (Ciddell & Alberts 2006). Solid chocolate is the type most frequently consumed now with a variety of additional ingredients used, for example nuts and raisins. Dark, milk and white chocolate are the three common types of chocolate, but white chocolate does not contain any cocoa mass, only the cocoa butter. Surveys have found that milk chocolate is the most popular in Europe, the UK and the USA. Also 87% of chocolate consumption in the USA is not through 'pure' chocolate, but that combined with nuts, caramel, wafers etc. (Vinson *et al* 2006).

3.2 Basic components and production process

There is a huge variety of chocolate available on the market nowadays, but they mostly consist of the basic ingredients cocoa mass, cocoa butter, sugar and milk. Today nearly half of the world's supply of cocoa beans is grown in the Ivory Coast, with the rest being from Ghana and other African nations, Indonesia and some other Asian nations, and a small percentage from Latin America (Ciddell & Alberts 2006*)*.

Ground-up cocoa beans are known as cocoa liquor and contain approximately 55% cocoa butter. Cocoa, cocoa mass or cocoa powder refers to the non-fat component of the cocoa liquor (Cooper *et al* 2008).

In the general process the cocoa beans are first fermented and dried, then undergo a process known as winnowing where the outer shell are removed leaving the cocoa nib. The nibs are ground up and separated into cocoa mass and cocoa butter. A small amount of the cocoa butter, sugar, vanilla and often an emulsifier lecithin are added to the cocoa mass, and undergo the conching process to remove bitterness and refine the texture

(Torres-Moreno *et al* 2012). Depending on country regulations and individual manufactures, conching can take up to 72 hours or it may not take place at all.

The proportion of each ingredient used and general processes must comply with regulations set by governing bodies in each country. For example the EU only allows up to 5% of the fat content to consist of non-cocoa butter, and it must be one of the substitutes they have chosen. Any nut varieties added to the chocolate must not exceed 60% of the total weight of the product, and any products that do not meet the criteria are not allowed to be sold as 'chocolate', 'dark chocolate' etc. There is a type of milk chocolate in the UK and Ireland that contains 20% cocoa solids, but as the minimum EU requirement is 25% cocoa solids it must be sold under the name 'Family Milk Chocolate' elsewhere in the EU (CBI 2012). The FDA in the USA defines 'milk chocolate' as containing as least 10% cocoa liquor, but interestingly has no definition for 'dark chocolate' (FDA 2012).

4. Cardiovascular Diseases

4.1 What are cardiovascular diseases?

Cardiovascular diseases (CVD) are major causes of death around the world, and are especially prominent in medium-low income earners. In 2008 17.3 million people died from CVD (30% of global deaths) and an estimated 23.6 million will die from it in 2030 (WHO 2012).

CVDs are diseases affecting the heart and blood vessels. There are several types depending on where the affected vessel supplies blood to and where the blood clot occurs, whether it existed at birth, and whether it was caused by infection. The most prominent of these is coronary heart disease (CHD), damage in the vessels supplying blood to the heart muscle.

There are many risk factors for CVD: smoking, obesity, diabetes and family history just to name a few. One of the underlying causes of CVD is atherosclerosis; the accumulation of modified low density lipoproteins (LDL) in the arterial walls causing plaque formation and blockage of blood

flow. Atherosclerosis can develop through oxidative stress, especially from LDL oxidation. LDL particles consist of around 2700 fatty acids, and about half of these are polyunsaturated and susceptible to oxidation. Oxidation is the addition of oxygen and greatly affects the activity of the particle. Oxidised LDL can inhibit the exit of macrophages while recruiting circulating monocytes, causing the formation of foam cells-sticky cells which accumulate inside the arterial wall. It can also reduce the bioavailability of NO causing endothelial dysfunction, and eventually cause strokes and heart attacks (Honarbakhsh & M. Schachter 2008).

4.2 Evidence of association of chocolate with CVD

A study looking at CHD found that consumption of chocolate five or more times a week, resulted in a 57% lower prevalence of CHD compared to those who never ate chocolate regardless of risk factors involved (Djoussé *et al* 2011). Another study focused on calcified atherosclerotic plaque in coronary arteries (CAC) in people who ate chocolate two or more times a week, once a week, one-three times a month and never. Results showed lower prevalence of CAC the more frequent chocolate consumption is. Lower systolic and diastolic blood pressures were also observed (Djoussé *et al* 2011).

A study using rabbits with artherosclerosis found that cocoa powder inhibited the development of artherosclerotic lesions (Kurosawa *et al* 2005). After six months, the area of artherosclerotic lesions was over 20% lower in rabbits that regularly consumed cocoa powder.

These are not the only examples, but the majority of studies are only carried out *in vitro* or *in vivo* on healthy individuals, so more research needs to be undertaken involving individuals with CVD or at least some of the risk factors to show the direct correlation.

5. Flavanols and their effects

5.1 *What are flavanols?*

The polyphenols is a large class of compounds that act as antioxidants. A subclass, the flavonoids have been associated with various health benefits, and the flavanols or flavan-3-ols in particular have been linked to cardiovascular health. Procyanidins are the main type of oligomeric flavanols and are composed of the monomers collectively known as catechin, shown in Figure 1. The catechin molecule consists of three rings, two of which are benzene rings (rings A and B). It has two chiral centres at carbons 2 and 3, meaning four diastereoisomers; (+)-catechin, (-)-catechin, (+)-epicatechin and (-)-epicatechin. The most common in cocoa are (+)-catechin and (-)-epicatechin and have been the focus of research.

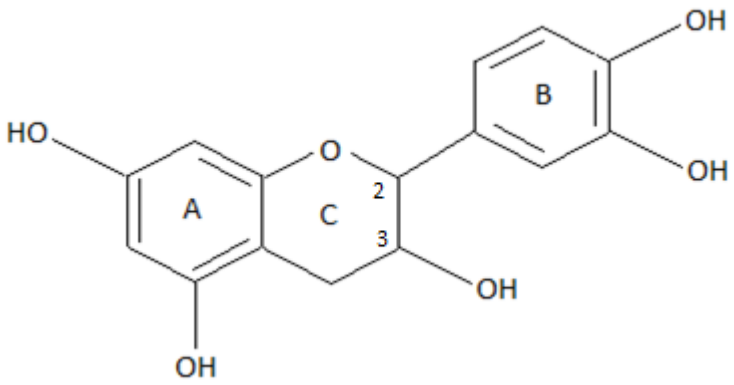

Figure 1: Basic structure of catechin (Tomoyo Oyama)

5.2 Antioxidant effect

All polyphenols have been found to have antioxidant activities *in vitro*, so now studies are being conducted to see whether this is the case *in vivo*. Various studies have shown chocolate to have antioxidant effects through the inhibition of LDL oxidation and increase in total antioxidant capacity (TAC).

The antioxidant effect was found to be dose-dependent, with a higher antioxidant capacity in chocolate with a higher cocoa solid content (Belščak-Cvitanovic *et al* 2011). *In vivo* experimentation has shown an increase in plasma antioxidant capacity after just a single dose of 100g of dark chocolate (Serafini *et al* 2003). Lettieri-Barbato *et al* (2012) found that the TAC of both dark chocolate and high antioxidant dark chocolate peaked 2 hours after ingestion. However the TAC of the high antioxidant dark chocolate remained relatively high even 5 hours after ingestion, while that of the other went down to zero. The inhibition of LDL peroxidation has been found in numerous studies, usually through the measurement of TBARS post-consumption (Mursu *et al* 2004*,* Mathur *et al* 2002*,* Vinson *et al* 2006).

Although the exact mechanism is unknown there are some possible explanations. Figure 1 shows that catechin molecules have a dihydroxylated B ring. This allows for the rapid donation of hydrogen to stabilise radical species (Engler & Engler 2006). Procyanidins (oligomers) are thought to accumulate at the liposome surface of cells and prevent the access of deleterious molecules inside the cell. The monomers catechin and epicatechin, and some dimers with epicatechin subunits are able to diffuse into the cells and affect enzymes and signalling cascades (Keen *et al* 2005).

5.3 Anti-inflammatory effect

Increasingly the endothelium is becoming considered as a vital organ in cardiovascular health. Dysfunction of the endothelium is often a result of the activation of proinflammatory enzyme systems through the production of reactive oxygen species (ROS) and radicals. Nuclear transcription factor (NF_KB) binds to the promoter regions of genes coding for pro-inflammatory proteins. Nitric oxide (NO) is a molecule required for vasodilation and normally inhibits NF_KB synthesis. However, oxidative stress and endothelial dysfunction reduce the bioavailability of NO, and intracellular ROS accumulate and NF_KB is activated. Catechin and epicatechin have been found to stimulate the production of NO, and suppress the production of proinflammatory cytokines while enhancing

the production of anti-inflammatory cytokines. They also inhibit NO-related nitration and oxidation reactions and prevent the activation of NF_KB (Engler & Engler 2006). Epicatechin in particular has been shown to have the more prominent role, and is also involved in the scavenging of free radicals and maintenance of the enzyme nitric oxide synthase for NO synthesis (Steffen, Schewe & Sies 2005).

A six week study on overweight adults found that consumption of cocoa led to an improvement in endothelial function, observed by measuring flow mediated dilation (FMD) *(*Njike *et al* 2011). Sweetened and non-sweetened cocoa were tested but the difference between the two were statistically insignificant. Heiss *et al* (2005) found that just a single dose of a high polyphenol cocoa drink was enough to increase levels of circulating NO and FMD, and studies testing single doses of dark chocolate also showed similar results (Hermann *et al* 2003*,* Vlachopulos *et al* 2005*).* The improvement is attributed to the flavanols which activate nitric oxide synthase, which then produce nitric oxide and induce vasodilation. Studies focusing on blood pressure showed correlations between chocolate consumption and decreases in both systolic and diastolic blood pressure (Ellinger *et al* 2012, Taubert *et al* 2003). This agrees with a study conducted on 14 healthy overweight and obese individuals, where consumption of dark chocolate containing 500mg and 1000mg of polyphenol lowered blood glucose levels and blood pressure (Almoosawi *et al* 2010). However there was not a significant difference between 500mg and 1000mg, suggesting that the effect plateaus after a certain amount of polyphenols, or after a particular blood pressure level. Chocolate may not lower blood pressure drastically, but it may be effective to combine chocolate consumption with other lifestyle changes such as exercise.

5.4 Anti-platelet effect

The formation of platelets is vital in the blood-clotting process when the blood vessels are damaged, but now is increasingly associated with heart attacks, strokes, and the progression of atherothrombosis. Plaques can build up in the vessels as a result of smoking, high blood pressure and

cholesterol, and the rupturing of these activates platelet aggregation and blood-clotting. Since there is no actual damage to the blood vessel, the blood-clot ends up blocking the blood flow (Ueno *et al* 2011).

A small scale study on 20 smokers showed that consumption of dark chocolate-but not white chocolate-reduced platelet activation (Hermann *et al* 2006). Cocoa is thought to reduce platelet aggregation by producing prostacyclin, a molecule that inhibits this process and induces vasodilation. NO (synthesised with the aid of catechin and epicatechin) is also involved in the inhibition process. This has been shown in *in vivo* studies where decreases in platelet activation and aggregation from cocoa consumption, correlate with increases in plasma epicatechin concentrations (Pearson *et al* 2002). Plasma prostacyclin levels were seen to increase by 32% following the consumption of high procyanidin chocolate (Schramm *et al* 2001). A single dose of semi-sweet chocolate chips was shown to increase the prostacyclin-leukotriene ratio and reduce platelet related haemostasis (Holt *et al* 2002). Leukotrienes are chemicals synthesised through the metabolism of arachidonic acid, and are important for antimicrobial defence. However they are proinflammatory, can stimulate platelet aggregation and induce vasoconstriction (Ding *et al* 2006). They are involved in the growth of artherosclerotic vascular lesions, and have been linked to strokes and myocardial infarctions (Peters-Golden & Henderson 2007).

5.5 Flavanol bioavailability

Not only is the mechanism of the flavanols unclear, the amount flavanols present in the final chocolate product is unknown unless each type is tested individually. This is because a significant amount of the flavanols can be lost during processing. A common method of producing cocoa powder is called Dutch Processing. It is an alkalising process that neutralises the acid and can result in a loss of up to 90% of the flavanols contained in cocoa (Miller *et al* 2008). An interesting study was conducted by Hurst et al. (2011) where the impact of common processes on flavanol stereochemistry of cocoa beans was observed. They concluded that (+)-catechin and (-)-epicatechin levels were reduced through fermentation,

drying, roasting and Dutch Processing, however there were increases in (-)-catechin. We cannot be sure whether the losses can be accounted for by the gains made, as the different ways in which each isomer works in unclear. It has been found however, that the preferred order of absorption is (-)-epicatechin > (+)-catechin > (-)-catechin.

A solution may be to omit these processes but it is not as simple as they improve palatability by removing the astringency, and increase microbiological safety through sterilisation. Currently there is work being put into retaining as much of the flavanol content as possible. Trials on a polyphenol-rich cocoa powder have been undertaken and shown positive results (Schinella *et al* 2010), and modification of the beans is also being looked into in Ghana (). It is also important to educate consumers as handling methods at home can affect flavanol levels. Stahl *et al* (2009) found that chocolate cake baked using baking powder retains a much higher level of flavanols compared to that made using baking soda due to the differences in the way they affect pH. Baking soda increases the pH of the cake more than baking powder, and a pH above 7.5 was noted as resulting in significant losses of flavanols.

6. Other effects

6.1 Lipid effect

Numerous studies observing the effects on lipid content have been conducted and a variety of results have been recorded. There are two major types of cholesterol; low-density lipoproteins (LDL) and high-density lipoproteins (HDL). LDL is the “bad” cholesterol as it can build up and stiffen the arterial walls, causing atherosclerosis. HDL is the “good” cholesterol as it carries cholesterol away from the arteries, lowering the risks of heart disease (American Heart Association 2012).

Wan *et al* (2001) in a four week study, found that individuals consuming regular amounts of either dark chocolate or cooca powder had a 4% increase in HDL on average compared to those without. Mursu *et al* (2004) found dark chocolate enriched with polyphenols to increase levels

of serum HDL by nearly 14%, and even dark chocolate by 11%, while white chocolate actually led to a slight decrease in HDL. Interestingly, they also found that all three types of chocolate led to a decrease in LDL peroxidation. Since white chocolate does not contain cocoa mass, this is thought to be due to the fatty acids present in cocoa butter. The inhibition of lipid peroxidation from monounsaturated fats, such as oleic acid found in cocoa butter has been found in previous studies (Eritsland 2000).

A study using rats found that regular intake of cocoa significantly decreased the levels of plasma cholesterol (Osakabe & Yamagishi 2009). The suggested reason for this is that the procyanidins in cocoa inhibit the intestinal absorption of cholesterol. Matsui *et al* (2005) observed an association between chocolate and weight loss, and suggested that flavanols supress the expression of LDL gene receptors and inhibit fatty acid synthesis. It is difficult to directly compare the studies however due to the different methods used. Greater increases in HDL levels may simply be due to the greater amount of cocoa administered for example.

6.2 Psychological effects

For some people eating chocolate can have positive psychological effects by lifting their moods. Dieting and cutting back on confectionery often leads to stress, and stress is a major factor in the development of cardiovascular diseases. Chocolate is often called a “comfort food” and can improve the mood immediately, possibly by directly affecting the brain neurotransmitters (Macht & Mueller 2007). A study on over 1300 men with an average age of 76 concluded that chocolate was associated not only with better health but also optimism and psychological well-being (Strandberg *et al* 2008).

6.3 Anti-cariogenic effect

Cocoa bean husk extracts (CBHE) have been found to have anti-cariogenic properties. CBHE are the by-products of cocoa processing and contain high levels of polyphenols. *Streptococcus mutans* and *Streptococcus sanguinis* are two types of oral bacteria that contribute to plaque formation and acid

production, subsequently leading to the formation of caries (Ferrazzano *et al* 2009). Cocoa polyphenols were found to inhibit biofilm and acid production in experiments conducted *in vitro* (Percival *et al* 2006*)*. *In vivo* experiments testing mouthwash containing CBHE found that they were effective in lowering the counts of Streptococci and overall plaque formation (Srikanth, Shashikiran & Subba Reddy 2008). It is important to note that cocoa mass found in commercial chocolate does not have the same properties, and eating large amounts of chocolate will not have the same effect. It is an interesting field of research however that is worth looking into. There are benefits not only from the dental point of view, but also from an economic point of view looking at the efficient use of by-products

6.4 Role of stearic acid

The fatty acids contained in chocolate are also a topic of interest, especially stearic acid as it may affect cholesterol and lipoprotein levels. Cocoa butter is contains on average 33% oleic acid (monounsaturated fat), 33% stearic acid and 25% is palmitic acid (both saturated fats) (Tokede, Gaziano & Djoussé 2011). Stearic acid has been found to have anti-platelet effects and reduce blood pressure according to some studies (Grassi *et al* 2005). This could be due to its chain length (stearic acid is 18:0 so a long-chain fatty aicd) and poor absorption, or it could be that it becomes desaturated into oleic acid -a fat that protects against CHD-upon digestion. Others claim that it has no such effects. They suggest that stearic acid is cholesterol neutral and has no effect on HDL or LDL levels (Kris-Etherton & Yu 1997). Studying the effects of stearic acid is difficult as it is not the only fatty acid present. It will interact with other fatty acids and may, as suggested, convert into other types. But either way there appears to be no research concluding that stearic acid has substantially negative impacts on health or cardiovascular health. Recently however, manufacturers are increasingly trying to substitute cocoa butter with healthier vegetable fats, but as mentioned before there are strict regulations regarding the types and amounts of ingredients used.

7. Looking into the future

Before any recommended daily intake values are given for chocolate it is necessary to become clear of their exact mechanisms and how they are metabolised. Commercial chocolate is high in calories, so will not have the same effects as polyphenol-rich chocolate made specifically for studies. A Swedish study involving middle-aged and elderly women found that consumption of chocolate 1-3 times per month or 1-2 times per week correlated with lower incidences of heart failure (Mostofsky 2010). However the incidences were higher in those consuming 3-6 servings per week and 1 or more servings per day, similar to the prevalence in those consuming no chocolate at all (the average serving size was 30g for those ≤61 years and 19g for those ≥62 years). This shows that excessive amounts of chocolate can have negative impacts, and further highlights the need for more information regarding effective dosages of flavanols and educating the public.

The experimental methods may need to be reviewed. For example there are a number of different ways to test the antioxidant activity of foods *in vitro*, and it has been found that they do not always give the same results (Pastoriza *et al* 2011). A standardised method to be used around the world will make comparisons easier, or at least some information clearly outlining each method would be helpful.

As mentioned before, the bioavailability of the flavanols are greatly affected by processing, and each manufacturer should be responsible for assessing their products. Manufacturing processes may need to be reviewed or changed depending on how much of the flavanols are lost.

Since chocolate varies so much around the world it may be useful to have some common standards. An indication of polyphenol content on the packaging for example, may help consumers during selection.

Currently manufacturers are trying to create new, healthier types of chocolate focusing on different aspects. As chocolate is still high in calories efforts are being made to reduce the sugar content. Cocoa beans containing greater amounts of polyphenols and cocoa powder that retains

high levels of polyphenols even after processing (Schinella *et al* 2010) are being looked into as well as cocoa butter alternatives and lower fat contents (Bootello *et al* 2012).

8. Conclusion

The vast amount of epidemiological evidence gathered from around the world means that we can be fairly confident in saying that chocolate contributes to cardiovascular health. Overall there appear to be three main ways in which flavanols in chocolate contribute to cardiovascular health:

1. They act as antioxidants by increasing the total plasma antioxidant capacity and reducing LDL susceptibility to oxidation.
2. They have an anti-inflammatory effect mainly through the stimulation of NO production
3. Platelet aggregation can be reduced contributing to healthy endothelial function.

There is also the possible contribution of stearic acid. Although it is becoming apparent that chocolate is beneficial to our health, it is important to remember that not all chocolate is the same. The origin of the beans and the production methods greatly affect the quality of the final product. The bioavailability of the polyphenols change depending on the production technique, and are actually often destroyed with processing. So the amount of cocoa contained in the final product cannot be used as an indication for the amount of polyphenols or flavanols it contains. Many popular chocolate bars have nuts, wafers, caramel etc. changing the amount of cocoa contained per weight, so it is difficult to give recommendations on how much we should be consuming. Also most of the research so far has only been conducted *in vitro*, or *in vivo* on a very small scale. Large scale studies concerning humans are usually through surveys, relying on individual honesty.

It is important to remember that although beneficial for cardiovascular health chocolate is by no means a cure for cardiovascular diseases. It is not always effective in those already with high risk factors, and greater lifestyle changes in diet and habits are required for improvement. It can also have a high sugar and caloric content so as with all things, should be taken in moderation.

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